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Systematic affinities of Aquifoliaceae and Icacinaceae from molecular data analysis

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RÉSUMÉ

SPICHIGER, R., V. SAVOLAINEN & J.-F. MANEN (1993). Affinités systématiques des Aquifoliaceae et des Icacinaceae par l'analyse de données moléculaires. *Candollea* 48: 459-464. En anglais, résumés français et anglais.

Reprenant une étude moléculaire des Celastrales à laquelle *Cornus* a été ajouté, cet article traite des affinités systématiques des *Aquifoliaceae* et des *Icacinaceae* avec les *Cornaceae*, les *Theaceae*, et les *Celastraceae*. L'examen des arguments morphologiques et la phylogénie construite à partir des séquences d'ADN du côté 5' de *rbcL* sont compatibles avec d'étroites relations évolutives entre les *Aquifoliaceae*, les *Icacinaceae*, et les *Theaceae*.

ABSTRACT

SPICHIGER, R., V. SAVOLAINEN & J.-F. MANEN (1993). Systematic affinities of Aquifoliaceae and Icacinaceae from molecular data analysis. *Candollea* 48: 459-464. In English, French and English abstracts.

Going back to a molecular study of Celastrales to which *Cornus* has been added, this paper questions the systematic affinities of *Aquifoliaceae* and *Icacinaceae* with *Cornaceae*, *Theaceae*, and *Celastraceae*. The examination of morphological arguments and phylogeny computed from *rbcL* 5'flanking DNA sequences are consistent with close relationships between *Aquifoliaceae*, *Icacinaceae*, and *Theaceae*.

KEY-WORDS: AQUIFOLIACEAE — CELASTRALES — Chloroplast DNA — CORNALES — ICACINACEAE — Molecular phylogeny — THEALES.

Introduction

The systematic placement of *Aquifoliaceae* and *Icacinaceae* is questioned. While HUTCHINSON (1973), TAKHTAJAN (1980), and CRONQUIST (1981, 1988) follow ENGLER's system (1964) by maintaining *Aquifoliaceae* and *Icacinaceae* among Celastrales, THORNE (1983, 1992) and DAHLGREN (1983) exclude them from Celastrales placement. EHRENDORFER (1991) replaces *Aquifoliaceae* among Cornales but does not mention *Icacinaceae* in his treatment in Strasburger 33rd edition. DAHLGREN (1983) replaces both *Aquifoliaceae* and *Icacinaceae* among Cornales. THORNE firstly (1983) placed the both families among Theales, then (1992) separates them by placing *Icacinaceae* among Cornales, and by leaving *Aquifoliaceae* among Theales. On the other hand, SAVOLAINEN & al. (submitted) demonstrate on the basis of a molecular analysis that the order Celastrales (sensu CRONQUIST) is polyphyletic and have to be divided into, at least, two major clades. The first clade, containing *Aquifoliaceae* s.l. (including *Ilex*, *Phelline*, and

Nemopanthus), is closely related to *Camellia* (*Theaceae*) and *Ikacina* (*Ikacinaceae*). The second clade, including *Euonymus* (*Celastraceae*), *Hippocratea* and *Salacia* (*Hippocrateaceae*) is related to *Euphorbiaceae*. Consequently, SAVOLAINEN & al. (submitted) reinforce THORNE's first placement (1983) of *Aquifoliaceae* and *Ikacinaceae* among Theales and out of the *Celastraceae-Hippocrateaceae* complex.

The purpose of this paper is to check by molecular analysis DAHLGREN's placement of *Ikacinaceae* and *Aquifoliaceae* among Cornales (1983) and THORNE's new classification (1992) of *Ikacinaceae* and *Aquifoliaceae*.

Material and methods

This present molecular analysis summarises that of SAVOLAINEN & al. (submitted) where details of procedures are exposed, to which the *Cornus mas* species is added, since it represents here *Cornaceae*.

Briefly, total DNAs of the samples were extracted, then an approximatively 900 base pairs (bp) chloroplastic DNA region was amplified by Polymerase-Chain-Reaction and sequenced. This region corresponds to the intergene region between the genes *atpB* (ATP synthetase beta subunit gene) and *rbcL* (rubisco large subunit gene), and the first 56 codons of *rbcL*. DNA sequences were obtained for 20 species and deposited in the EMBL/GenBank (except for *Cornus*). The sequences were aligned manually, yielding a 1353 bp long matrix, whose 172 bp were removed from the analysis because no satisfactory alignment could be obtained or because of nucleotide indetermination. A cladistic approach was performed by analysing this reduced matrix using the maximum parsimony method, which attempts to minimize the number of character states changes (here DNA mutations) among the evolutionary units (taxa). For that matter, we used the heuristic search option included in the PAUP software of SWOFFORD (1991). In order to improve the robustness of our trees, we used the resampling method of Bootstrap (1000 heuristic bootstrap replicates performed) also included in the PAUP software.

Taxa

We added *Cornus mas* L. (*Cornaceae*, sample coming from the Botanical Garden of Geneva) to the 19 species used by SAVOLAINEN & al.: *Euphorbiaceae* (*Euphorbia esula* L., *Euphorbia dulcis* L., *Mercurialis annua* L.), *Rhamnaceae* (*Rhamnus cathartica* L., *Colletia armata* Miers), *Rosaceae* (*Rosa damascena* Mill., *Geum coccineum* Smith cv. *Borisii*, *Malus × domestica* Borkh.), *Aquifoliaceae* s.l. (*Ilex aquifolium* L., *Ilex pernyi* Franchet, *Nemopanthus mucronatus* Druce, *Phelline comosa* Labill.), *Ikacinaceae* (*Ikacina mannii* Oliver), *Celastraceae* (*Euonymus bungeanus* Maxim., *Euonymus maackii* Komarov), *Hippocrateaceae* (*Hippocratea richardiana* Cambess., *Salacia pallescens* Oliver), *Theaceae* (*Camellia sinensis* Kuntze), and *Magnoliaceae* (*Magnolia liliflora* Desr.).

Results

Two most parsimonious trees were obtained by the heuristic search option of PAUP (SWOFFORD, 1991), whose length is 1611 steps, consistency index is 0.75 and homoplasy index 0.25. These trees were rooted by *Magnolia*. They differ only by the position of *Ilex pernyi* which is more related to *Ilex aquifolium* in one tree, and to *Nemopanthus* in the other one. One of these trees is presented in figure 1. To avoid "excess of parsimony" the same analysis was performed in authorising trees with 3 extra steps. In this case, 35 trees were obtained, and the topology of the strict consensus tree is not drastically changed (data not shown). In the molecular tree presented in figure 1, branches whose bootstrap values are higher than 95% are considered as significant. This is the case of all branches except for the position of *Cornus* (the lower bootstrap value of 53% means that its place-

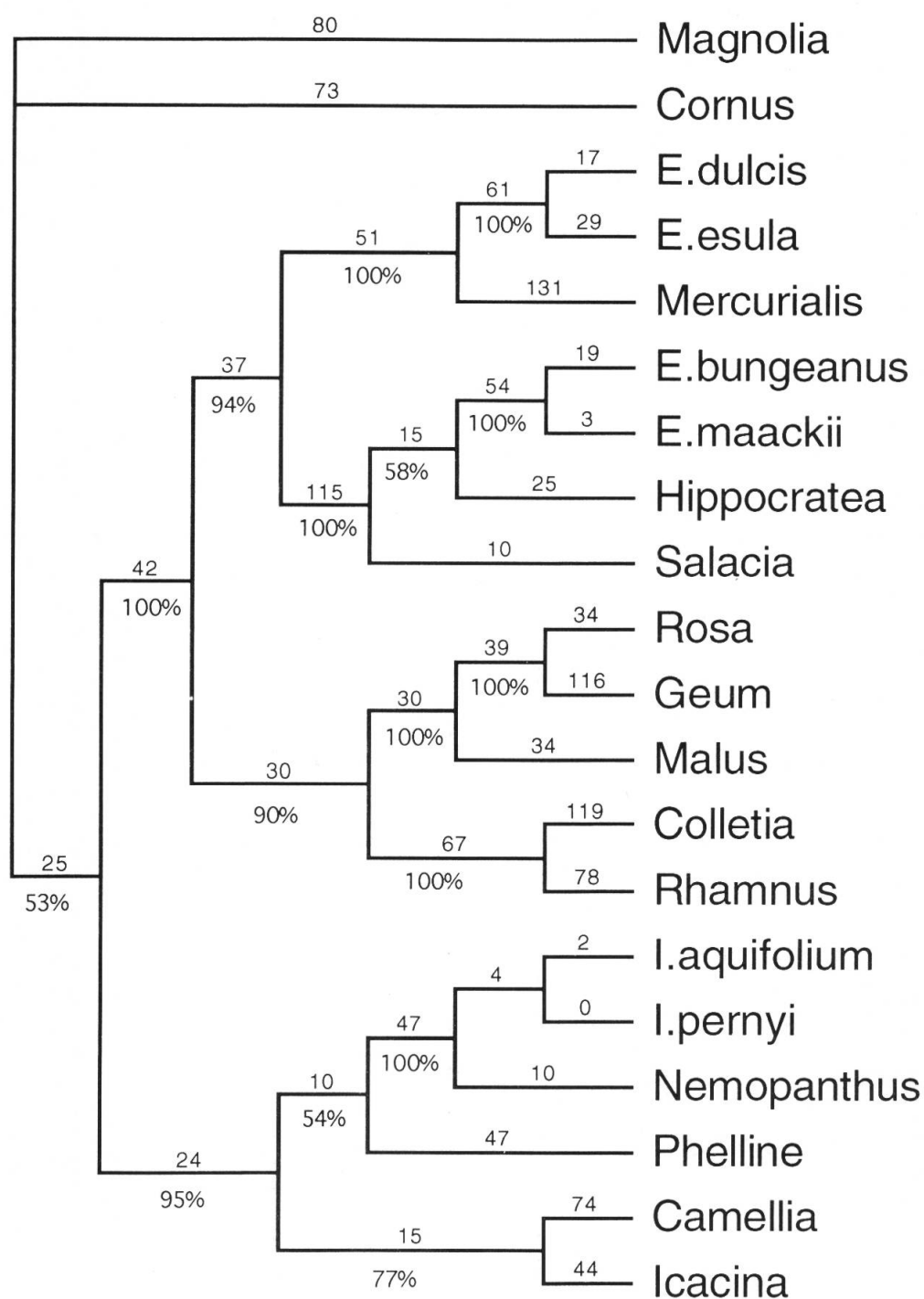


Fig. 1. — One of the two most parsimonious trees obtained from molecular data (length 1611 steps, consistency index 0.75, homoplasy index 0.25). The numbers of steps (mutations phylogenetically informative) are indicated above the branches and the percentage of bootstrap resampling are indicated below the branches (groups present in at least 95% of the trees can usually be considered as significantly supported, 1000 bootstrap resampling were performed). The tree was rooted by *Magnolia*. two major clades are well defined, one containing *Aquifoliaceae* s.l. (*I. aquifolium*, *I. pernyi*, *Nemopanthus*, and *Phelline*), *Icacina-ceae* (*Icacina*), and *Theaceae* (*Camellia*), and the other containing *Euphorbiaceae* (*E. esula* and *E. dulcis*), *Rosaceae*, *Rhamna-ceae*, *Celastraceae* s.l. (*E. bungeanus*, *E. maackii*, *Salacia* and *Hippocratea*). *Cornus* is the sister group of the two clades cited above.

ment is not defined, certainly because it is not closely related to the analysed species, see discussion), *Hippocratea* (58%) (which could be directly related to *Salacia* among *Hippocrateaceae*), and *Phelline* (54%), *Camellia* and *Icacina* (77%) (the relative relationship between these three latter genera is not clearly defined in this analysis, however, the 95% bootstrap values indicate that these genera are very close to *Ilex* and *Nemopanthus*). However, the 95% bootstrap values indicate that these genera are very close to *Ilex* and *Nemopanthus*. Thus, the addition of *Cornus* to the trees obtained by SAVOLAINEN & al. (submitted) does not modify their topology because *Cornus* is not related to these species, and the same two major clades are reobtained. *Cornus* is the sister group of these clades in the most parsimonious trees (see Figure 1). In the 3 extra steps trees, *Cornus* is, in some cases, related to the clade *Aquifoliaceae-Phellinaceae-Icacinaceae-Theaceae*, which always remains monophyletic (data not shown).

Discussion

After having demonstrated the polyphyly of Celastrales (sensu CRONQUIST) by a phylogenetic analysis based on molecular data (SAVOLAINEN & al., submitted), we discuss in this paper the relationships of *Aquifoliaceae* and *Icacinaceae* with Celastrales, *Theaceae* and *Cornaceae*. These relationships are shown on the cladogram of Figure 1 and are discussed in regard of morphological considerations.

The Figure 1 draws two major clades: the first one grouping *Aquifoliaceae* s.l. (incl. *Phelline*), *Icacinaceae*, and *Theaceae*; the second one with *Rosaceae*, *Euphorbiaceae*, *Celastraceae*, *Hippocrateaceae* and *Rhamnaceae*. These results are consistent with THORNE (1983, 1992), and DAHLGREN's (1983) opinion of deleting *Aquifoliaceae* and *Icacinaceae* from Celastrales (sensu CRONQUIST). *Icacinaceae*, *Aquifoliaceae* s.l. and *Theaceae* (*Camellia*) appear on one hand as closely related to each other on our cladogram, and are remote from *Cornaceae* (*Cornus*). Consequently, *Icacinaceae* and *Aquifoliaceae* should neither be placed into two different orders as far as Cornales and Theales, nor placed both together among Cornales. This result is neither consistent with DAHLGREN's placement (1983) of *Aquifoliaceae* among Cornales, nor with THORNE's one (1992) of *Icacinaceae* among Cornales. Our cladogram corroborates THORNE's classification of 1983 which places *Aquifoliaceae* and *Icacinaceae* among Theales and excludes a close relationship with *Cornaceae* and *Celastraceae*. THORNE (1983) expressed strong confidence in his placement of *Aquifoliaceae* among Theales (superorder Theiflorae), but a more limited confidence in the placement of *Icacinaceae* in that order. In his more recent classification (1992), this author transfers *Icacinaceae* from Theales to Cornales, but confirms the placement of *Aquifoliaceae* among Theales. However, our results invalidate his new point of view about *Icacinaceae*.

Our own experience of *Aquifoliaceae* and *Icacinaceae* in the field, based on morphological and macroanatomical characters, makes our opinion favorable with the classical placement of both families in one and same order. In the field, neotropical trees of *Aquifoliaceae* and *Icacinaceae* have often the same appearance and can be mistaken one from the others. The similarity of some vernacular names is consistent with close relationships (ORTEGA-TORRES & al., 1989; SPICHIGER & al., 1990) and proves that native people see these two families as closely related one to another.

Studies of wood and leaf anatomy (BAAS, 1973, 1975) allow to place *Icacinaceae* and *Aquifoliaceae* together. Nevertheless authors differ in the placement of both families since BAAS (1975) leaves them in Celastrales whereas DAHLGREN (1983) replaces them in Cornales. According to DICKINSON & BAAS (1977), the inclusion of *Aquifoliaceae* and *Icacinaceae* in the order of Celastrales is doubtful since there is a large number of anatomical similarities between *Aquifoliaceae*, *Icacinaceae*, and *Theaceae*.

According to KELLER (1992), *Icacinaceae*, *Aquifoliaceae* and *Theaceae* generally have monopodial branches, alternate phyllotaxy, and entire or denticulate leaves. These families have generally exstipulate leaves, but *Aquifoliaceae* may be stipulate. *Cornaceae* (*Cornus*) present distinct features as sympodial branches and opposite leaves. Nevertheless, many species of *Celastraceae* (sensu CRONQUIST) present very similar features to those of *Aquifoliaceae*: monopodial

branches, alternate leaves with denticulate margins. These morphological affinities make sometimes difficult the separation of both taxa and have been used — among others — to place *Celastraceae* and *Aquifoliaceae* closely together.

The flowers of *Icacinaceae* and *Aquifoliaceae* are dialypetalous or gamopetalous (sometimes only slightly), isostemonous with alternipetalous stamens, and hypogynous. They differ from *Cornaceae* (*Cornus*) which are epigynous, from *Theaceae* (*Camellia*) which are polystemonous with a spirally arranged perianth, and from *Celastraceae* (*Euonymus*) which present a thick intrastaminal disc. The unitegmic ovules of *Aquifoliaceae* and *Icacinaceae* might bring these both families together among Cornales (DAHLGREN, 1975). This opinion is opposed by DICKINSON & BAAS (1977) because of too many other morphological and anatomical disparities. Furthermore, some genera of *Theaceae* present also unitegmic-ovuled members (DAHLGREN, 1975).

On the only basis of floral characters, *Aquifoliaceae* and *Icacinaceae* seem closer to *Celastraceae* (*Hippocrateaceae* excluded) than *Theaceae* or *Cornaceae*. It is the reason for the traditional placements of Engler, Takhtajan, and Cronquist. Nevertheless the wanting disc and the unitegmic ovules differentiate strongly *Aquifoliaceae* and *Icacinaceae* from *Celastraceae*. The wanting disc and the alternate exstipulate leaves associate *Aquifoliaceae*, *Icacinaceae* and *Theaceae*, whereas the unitegmic ovules associate the two former families with *Cornaceae*. It is noteworthy that the cladogram (Figure 1) drawn from molecular analysis shares the disciflorous taxa from the non disc-bearing ones.

Studies on pollen morphology by LOBREAU-CALLEN (1969, 1975, 1977) separate *Celastraceae* and *Hippocrateaceae* which have colporate apertures, from *Aquifoliaceae* and *Icacinaceae* which miss this structure and are closer to *Theaceae* pollen-type.

Our molecular analysis is another argument for the placement of both *Aquifoliaceae* and *Icacinaceae* closer to *Theaceae*. Because morphological convergences occur often in plants, molecular phylogenetics should be useful to decide between the different opinions based on morphology.

Thus, in our present study, we obtain results which are more consistent with THORNE's classification of 1983 and with DICKINSON & BAAS (1977) point of view, than other systematic treatments. In order to define the phylogeny of *Aquifoliaceae* and *Icacinaceae* more accurately, other families have to be added, and other parts of the genome have to be analysed. Particularly the coding sequence of *rbcL* is currently analysed, because more and more of this sequence is available and a new approach of the systematics of Angiosperms is now possible. In the future, this kind of work would permit a sharp analysis on the evolution of the above discussed morphological characters.

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